PHYSIOLOGICAL (PARANECROTIC) HYPOTHESIS OF THE MUTATION PROCESS

- USSR -

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FOREWORD

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The problem of the origin of something new in the organism is one
of the basic mysteries of nature to the researchers of natural science.
If it were possible to determine to the end the development of a new
function or feature in ontogenesis, that is, to learn the mechanics of
the process of the formation of the organism as a whole system, there
would be no limit to the might of man in the transformation of nature:
the biologist would become "the designer" of new living substances--
forms necessary for human life. The aspiration to achieve this high
goal is that force which drives the contemporary researcher of natural
science, and in particular, the Soviet biologist.

In this respect Charles Darwin made the first and important in
principle step, having proved the variability of species. He explained
the driving forces of the history of the organic world. However, the
Darwin epoch could not at the same time resolve also the second problem
which logically stems from his evolutionery teachings, that is the
method of the active transformation of nature. Our society, therefore,
highly values the works of those who are striving with their investi-
gation to penetrate the secrets of the development of the organism,
the form developing processes. The names of I. P. Pavlov and I. V.
Michurin became the standards bearers of contemporary biology.

What then is the decisive moment when establishment of new in
ontogenesis is perceived?

The main link in this process is the clarification of the causes
of variability. Darwin begins the first chapter of the "Origin of
Species" by presenting his theory of the "causes of variability." He
solved this problem only in a general principal form: "two factors must
be distinguished--the nature of the organism and the nature of condi-
tions." In this solution, the monism of the entire evolutionery theory
of the driving forces of the development of the organic world is mani-
fested.

Although, the formula for the solution was given almost a cen-
tury ago, the problem of the cause of variability still remains un-
solved, for the approach to the solution of this problem was one-sided.
Some preferred to regard the "nature of conditions" (followers of
Lamarck, ectogenetists) as the source of variability; others, on the
other hand--as the "nature of the organism" (autogenetists). The entire
Post-Darwinian period to our days is characterized by a struggle between

these two trends. Both currents in the course of their investigations accumulated considerable factual data which to these days is waiting for a proper analysis and summation.

It became apparent that neither of the mentioned two factors ("the nature of the organism" and "the nature of conditions") can be paramount in the determination of the causes of variability. The role which these two factors play in the process of variability changes. In relatively constant conditions the environment may be the basic driving force of mutation. The advocates of both trends could, therefore, present arguments which favored one or the other factor.

Darwin has indicated a number of times that the leading cause of variability is a change in the conditions of existence of the organism. Even any kind of a slight change may be sufficient to induce variability. In the course of time, however, the organisms may become adopted to some changes and become less changeable. In these thoughts is the key to the understanding of the interrelation of the nature of the organism and the nature of conditions. A change in conditions presents new demands from the organism, but if the changed conditions act monotonously in the course of the development of the organism or a number of generations, the organism becomes adopted to these conditions and the new conditions become a normal part of its existence. The organism can react to the changing conditions by two methods: first, by way of the direct adaptation in ontogenesis (ontogenetic adaptation) if the new conditions do not overstep the limits of the historical (inherited or genotypical) possibilities of the organism; second, by way of selection of separate (single) individual species which possess the capacity to adapt themselves to the new conditions. For the unadaptable representatives of this specie the new conditions may become a source of variability. The reason for variability are those same conditions of existence or organisms which are capable of changing the direction of the tempo of selection. Selection and variability--these are different sides of the same process, the driving force of which is the change in conditions. It is this that changes the intensity (tempo) and direction of the selection in the population of the specie and simultaneously, as if automatically, increases the general frequency of the origin of inherited variables. In carrying out this thought further, it is possible to arrive at an important, principal conclusion, that is: the effectiveness of selection is determined by the character of changed conditions in the direction necessary for selection; selection in constant conditions will sooner or later prove to be little effective. The change in conditions is a kind of a provocational factor which intensifies accidental variability, and if it is carried out in a direction necessary for selection, success in selection may be attained in a shorter period of time. As an example, let us remember the forgotten experiments by Mon'ye to which Darwin frequently referred. These experiments concerned the transformation of winter wheat, barley, and other grain crops, into spring crops and the reverse (from spring to winter) in a period of

three years. Similar examples may be considerably multiplied at the present time. In these experiments the question is not only that of selections of "pure lines" among the heterogenous population, but of the unfavorable change of conditions which increases variability and thereby creates a base for the activity of selection. On the basis of the data on the effect of external conditions on the mutation process it may be said that with a change in the conditions of the environment, the tempo of inherited variability is intensified and that selection may be effective within the limits of the pure lines, as well as in the heterogennous population.

If we now turn to an analysis of the other factor of variability-- the nature of the organism, we discover in evolution a regular line common to all organisms: the development of adaptable reactions in the organism which guarantee to the organism adequate reactions to the change in the conditions of the invironment. I. I. Schmal'hauzen (1946) very well formulated this proposition: "In the process of evolution the or-ganism becomes emancipated from the factors of the external environment (the author has in view their change--M. L.), becoming stabilized in its vital processes and in its more autonomous individual development. We may speak about the autonomization of ontogenesis as one of the direc-tional (in general) processes in the evolution of the organic world." The relative autonomization of the organism in evolution emerges as a factor which limits and controls the effect of the changing conditions on the process of variability; it is in this primarily that the nature of the organism is manifested. In those cases in which the autonomiza-tion of the organism is inadequate, the action of the change in conditions finds its application. In order, therefore, to study the effect of the conditions of the environment on the process of variability, it is nec-essary to determine even if only in general the reactions of the organism which make it possible for it to control the external factors which act on it. To these reactions belong first of all the ancient property of the living substance of the cell to adopt itself to the monotonous or rhythmical (cyclical) repeating action of the same conditions of the environment in ontogeneses; second, the adaptive reactions of the organism as a whole which are controlled by the activity of the nervous system. A number of other moments which control the effect of external factors of the environment on modifications in the cell will be noted later.

Thus, in summing up all that has been said, it is possible to formulate a common principle of the causes of variability: extreme or even small deviations of the conditions of development from the physi-ological optimum which take the organism beyond the limits of the possi-bility of adequate (adaptive) reactions to a change in conditions form one of the basic sources of its variability. These same conditions, if they act on a number of generations, modify the trend and tempo of selection.

It was established that for modificational variability "the digression of the environment from the optimal values increases variability" (Yezhikov, 1933). An analysis of mutational variability? A rise in the modificational variability under unfavorable conditions is easy enough to comprehend if it is considered that it reflects a multiplicity of adaptive reactions of the organism to concrete conditions of the environment, and is the final result of the realization of the genotype in the course of ontogenesis. Different organisms in a population differ in their resistance to a change in conditions. Resistance to unfavorable conditions in the environment is secured by the ability of the organism to adopt itself to these conditions; extreme deviations of the environment from the optimal value produce a genotypical variety. They are the provocational element of the potential capacity of the hereditary nature of the organism. Optimal conditions of development conceal the entire gamma of the adaptive capacities of the organism and provide a neutrally expressed index which like a ray of light can be broken down only when it is passed through a prism.

Thus, in order to comprehend the mutual relations of the nature of the organism and the nature of conditions it is necessary, first of all, to appraise the ability of the organism and its tissues to adapt themselves to the conditions of the environment, for it is the latter that controls and limits their influence. The phenomenon of the functional adaptability of the organism, and its separate organs and tissues to monotonously acting environmental conditions is well known. Examples of such adaptation of the human organism are the accomodation of the eye, and the habitual use of narcotics and drugs upon the prolonged usage of one and same prescription. Even such a strong antibiotic as penicillin probably can lose its antibiotic properties upon prolonged usage, for the microorganisms which produce diseases possess a greater capacity for adaptation than do the human tissues. In such cases drugs may have a contrary effect. In citing these reflections, I would like to emphasize the exceptional significance of the adaptation problem to medicine as well.

However, the well known investigations of this subject have sought only to clarify functional adaptation without touching on the changing properties of the living substance of the cell in the process of adaptation. It is apparent, at the same time, that only the investigation of the living substance of the cell may throw a light on the mechanism of the organism's adaptation to the factors of the environment.

One of the first attempts in this direction was made by Lobashev and Korenevich who applied the method of vital staining of tissues of the organism. D. N. Nasonov, V. Ya. Aleksandrov (1940) and their co-workers revealed that the increase in the sorption properties of living tissues of a number of stains may serve as an indication of paranecrotropic changes of the cell at the basis of which is the fact that they permit

a reversible denaturation of the proteins of the protoplasm. A change in the quantity of stain adsorbed by the tissue serves as a well known indication of the degree of injury suffered by the cells. Using this comparative method, it is possible to evaluate the sensitivity of the cells of different organisms to one or another influence. In our experiments, one group of tadpoles was adapted to high temperature while another group was kept in a low temperature. After a certain time of adaptation to different temperatures, both groups of the tadpoles in a weak neutral solution were subjected to the effect of a high temperature. Had the preliminary adaptation to different temperatures produced changes in the protoplasms of the cells, then at the moment of the simultaneous action of high temperature, on them, the tadpoles which were adapted to high temperature should have tolerated that temperature than did the unadapted tadpoles. The experiments fully confirmed the expectations. The cells of the unadapted tadpoles absorbed the stains to a considerable greater degree than did the adapted. Analogous experiments with a frog's muscles also confirmed this phenomenon which we termed "substantial adaptation." These experiments make it possible to speak not only about functional adaptation but also about substanial adaptation. The living substance of a cell can accustom and adjust itself to a definite rhythm of living conditions.

These investigations have shown also that at the same time the threshhold of injury is displaced and is preserved for some time, even after the organism is transferred to other conditions. The importance of these phenomena for the comprehension of the causes of variability are apparent. They can clarify for us some phases of the process of acclimatization, immunity, and so forth. A proof of substantial adaptation may serve as a firm confirmation of the broad meaning of I. V. Michurin's teachings and the investigations conducted by the T. D. Lysenko school in regard to the modification of the properties of plants in vegetative hybridization. This makes it possible to assume that the phenomena which they discovered may be applied to animal objects in transplantations and combinations (chimeras).

However, the basic meaning of the experiments which we conducted is in the fact that they confirm the changes of some properties of the living substance of the cell in changing conditions. The process of adaptation is found to be connected with changes in the substrata of the cell. Therefore, every change in conditions should be regarded as an "unfavorable" moment which leads to a change in the structure in the protoplasm of the proteins. It is in this, indeed, that we find the basic meaning of the changing conditions in the process of variability which provides material for selection.

The experiments conducted by Kozhanchikov (1940), Lozina Lozinskiy (1943), Melanbi (1939), Wells (1935) and others fully confirmed the fact that a change in conditions leads to a change in the reaction of the living substance.

Kozhanchikov studied variability in some of the lepidoptera and found that the indices which are connected with the expenditure of energy in the period of growth are characterized by a minimal variability during their optimum of development and a high--in extreme conditions. At the same time he proved that at extreme conditions of the optimum a rise in oxygen absorption may be noted. A fact which is of no lesser interest was observed in the wintering caterpillar of the apple worm by Lozina-Lozinskiy. He carried them from different winter temperatures to different experimental temperatures and measured their respiration. It was interesting that a decrease in intensity of respiration took place at a temperature in which the object was kept before the experiment. The greater the contrast in temperature changes the greater was the rise in oxygen absorption. The rise in oxygen absorption which takes place as a result of extreme deviation from the optimum and changed conditions indicates that substantial changes, which apparently increase metabolism in order to maintain the native proteins at a certain level, take place at the same time.

When an analysis of the genetic data on the origin of mutations and other intracellular changes is made from this point of view, we obtain a picture which is analogous to that which was noted when the reasons for modifictional variability were investigated. The less the organism finds itself adapted to some of other factors of the environment, the greater will be the effect of these factors on the process of mutational variability, if these factors can freely reach the cell.

I shall cite several examples of data which were not regarded from this point of view. Colchicine is an alkaloid which inhibits the formation of the spindle of mitosis in the cell. The effect of colchicine on more than 300 types of higher plants was investigated to the present time, and in all cases it produced some or other types of injurious (colchicined) effects. However, the effect of colchicine when applied in concentrations of 0.01 to 0.1 percent and even 1.0 percent to four types of Colchicum specie, a plant from which the alkaloid is isolated, for a period of 6 to 30 days, failed to produce the expected results. It should be mentioned at the same time that the percentage content of the alkaloid in the plant itself is smaller than the dose with the help of which an attempt was made to obtain a colchinized effect. Obviously the cells of the plant which contained colchicine did not produce a pathological picture in response to its effect, having adapted themselves to the alkaloid and its action. A similar explanation may be given for the increase in the percentage of the crossing of chromosomes during the extreme temperatures of the development of drosophila in the experiments carried out by Plu (1917).

The experiments carried out by A. I. Zuytin in which he investigated the effect of contrasting changes in temperatures on the rise in the frequency of the origin of mutations is of great interest as an illustration of the situation which developed. He proved that if the temperature regime for the drosophila is changed rapidly and a number of times, the number of developing mutations is increased. He explains this

phenomenon as a result of the direct effect of the contrasting change in temperature. It would be more correct, however, to ascribe these results to the injury of the cellular substance. Substantial adaptation is inhibited by the change of temperature. This is corroborated by the following: special experiments in which the effect of constant and fluctuating temperatures on the development of sex cell deliberately injured by X-rays was clarified, were conducted already in 1935-1936 (Lobashev, 1938); a calculation of the mutation changes which took place under the influence of the X-rays in these experiments served as an indicator of the survival of the sex cells. The immature cells which carry the mutations are sensitive to conditions unfavorable for development, and can, therefore, conveniently be used to calculate the effect of the environment on a change in cell reaction. In these experiments the homogenous larva of the drosophila were irradiated with X-rays and then divided into two groups: one group developed to (imago) in a constant but unfavorable to development temperature (30 degrees C), while the other in temperatures fluctuating between 13 to 25 degrees C. The latter temperatures are almost within the limits of the physiological optimum: the lower limit is about 15 degrees; the upper limit of development—30 degrees. It could be expected at first, that the number of immature cells with mutations would be smaller when developed at a 30 degree temperature than when developed at temperatures of 13 to 25 degrees C. The results of the experiments, however, produced a reversed picture. The number of mutations which developed at a constant although unfavorable temperature of 30 degrees was 3.45 percent, while that in fluctuating temperatures—1.70 percent (1.75 - 0.67). The fluctuations of temperatures which had an inhibiting effect on the adaptation of the sex cells to a specific regime of developing conditions, had a greater harmful effect on the survival of the cells than did constantly acting but unfavorable temperature (30 degrees C). Thus, in occasional cases, unfavorable conditions if they are constant during the development, are less harmful to development than are frequent changes of favorable and unfavorable regimes. The supposition was made then that in the development at constant temperature an "adjustment" of the organism or its sex cells takes place in ontogenesis to a specific temperature regime, and that frequent changes in temperature displaces and disturb this adjustment and inhibits organism adaptation. The reason for the depression is the large expenditure of energy, a reason which coincides with the mentioned data of Lazina-Lozinskiy and others in regard to a rise in metabolism when the temperature regime is changed.

Direct experiments were carried out by us recently. These experiments clarified the significance of the organism's adaptation process as a factor controlling the effect of external conditions on intracellular and substantial processes. Two lines were evolved from a normal laboratory line of drosophila. One of these continuously developed at a temperature of 30°C for four generations; the other—during four generations at a temperature of 15°C. Had the drosophila lines adapted themselves to various temperatures in the course of these generations, with

high and low temperatures acting on them simultaneously, it could be expected that the heat-preferring line (first) would better tolerate high temperatures and worse, low temperatures; while the cold-preferring line would conduct itself in a different manner.

Tests conducted to determine the tolerance of the development process to high temperature established that survival in the heat - preferring line was 56.6%, and in cold preferring--38.1%. When the representatives of these same lines were subjected to the effect of 0° temperature for a period of four to six hours, a striking difference was noted: survival in the first line amounted to only 0.9 to 0.8%, and in the second line--to 50.8 to 55%. Thus, in this last experiment adaptation provided a difference in tolerance to low temperature of more than 50 times.

A similar picture was obtained when an analysis of the intracellular changes (nonsegregation of X-chromosomes) in the sex cells of representatives of those lines which developed in a high temperature was made. The heat-preferring males when developing in high temperature provided 0.056 percent of nonsegregation of chromosomes, while the cold-preferring at the same temperature--0.161 percent. These experiments have, for the first time, proven that the "mutation process may be regarded as an adaptation process in specie formation, the speed and direction of which is controlled by the degree of the adaptation of the organism, and the separate stages of its development to conditions of existence" (Lobashev 1938). This proposition was advanced on the basis of preliminary considerations; it has been now fully confirmed by experimentation.

On the basis of what has been already said we come to the conclusion that one of the principal causes of mutational variability is the injurious effect of external factors on the sex cells. The extent of the injury caused by one or another factor is controlled by the sensitivity of the organism as a whole and its sex cells, while sensitivity is conditioned, primarily, by the adaptation processes.

In concluding this section it is necessary to once more emphasize our basic idea, that the processes of selection and the dynamics of mutational variability are interlinking, mutually connected phenomena, conditioned by a change in the relations between the organism and the environment. The change in conditions is the driving force for the organism in the specie forming process.

It is not possible to discuss here in detail the interrelations of the processes of variability and selection which together with heredity are the basic factors of evolution, according to K. A. Timiryazev. We have attempted to demonstrate here only the organic connection between selection and the process of variability. Changes in the direction of selection or a speed up in its tempo are simultaneously accompanied by a compulsory increase in mutational variability. A change in conditions is at the basis of the mutual relationship of these two processes.

In the light of these considerations it becomes important to elucidate the effect of a change in conditions on the process of the origin of mutations.

III

When the experimental data on the mutation process are organized into a definite system, it may be said that mutation develops as a result of the injurious (sublethal) action of agents which are completely different in their nature. Mutation takes place as a result of the extreme deviations from the optimum of the conditions of the organism's development, deviations which take it beyond the limits of adequate, that is adaptable reactions to a change in conditions.

Mutation is caused by the action of radiation energy (X-rays, ultraviolet rays, radium rays, and so forth), high [sic] and negative temperatures (minus 5 to minus 12 degrees C), contrasting changes of the temperature regime, the action of chemical substances: iodine, ammonium, yperite, potassium permanganate, arsenic, copper sulfate, lead nitrate, and by the effect of asphyxiation, aging seeds, deficit of some salts in plant nutrition, and others. It is evident from the list of the mentioned agents of mutation that mutation is not the result of a specific reaction of the gene to the action of some specific group of agents. The agents differ in their chemical as well as physical properties. Most of them are not normal to the organism and are beyond the limits of the historical adaptation of the organism to these agents. Neither can the quantitative difference in the effectiveness of the agents acting on mutation serve as a cue of the specificity of their effect on the modification of the gene, as their paths and positions in the organism differ. We shall speak about this further.

An injurious effect on the cell is a feature which is characteristic and common to the action of all known agents of mutation. Numerous cytological investigations indicate that one part of the injured cells sooner or later dies, while the other repairs the damage. In sublethal effects, exaggerated forms of modification are noted; these become still more noticeable upon the recording of the developing mutations and the observation of the pathological pictures in the cell. This, however, does not provide a basis for the rejection of the fact that even weak effects (small changes of conditions) are capable of producing intracellular changes. Between the physiological "quiescence" and the irreversible state of the cell (death) there are a number of uninterrupted stages of cell irritation, the result of the continualy intensifying action of the agent. Vved'ensky (1901) in his well known studies on parabiosis has for the first time provided a basis for nervous phase transmission in the development of irritation, such transmission depending on the force and frequency of the irritant, and the reversibility of the parabiotic state of the nervous system produced by the action of narcotics; this law was demonstrated further when various agents were applied. On the basis of these teachings, Ukhtomskiy writes

that the "nerve dies only after going through a stage of irritation. Death from irritation differs only by reversibility. Death is irritation which becomes irreversible." (Collected Works, Vol. IV, p. 15). Therefore, when speaking about injury as a state which contributes to the origin of mutation, we have in view under the term "injury" the extreme degree of reversible irritation. Therefore, injury cannot be regarded literally as a pathological phenomenon; it is a compulsory stage, or to be more exact--an expressed reaction of the living system to an unfavorable change in the conditions of the environment. In the evolutionary aspect, a reversible injury may be regarded as one of the basic adaptive reactions of the cell which permits it to parry accidental fluctuations of the environmental factors.

The investigations which were conducted by the Nasonov school, already friefly mentioned, make it possible to tie in the changes in the functions of the nerve fiber, and in the sex and somatic cells with the modification of protoplasm proteins in reversible injuries. Nasonov and Aleksandrov established that when injurious doses act on the cells of animals and plants, regular reversible changes in some of the properties of the cytoplasm can be noted, if the action of the agent did not go too far. These changes are characterized by an increase in the sorption properties of the cytoplasm to a number of stains, a decrease in the dispersion of the colloids, an increase in the viscosity of the cytoplasm of the cell, and others. Nasonov and Aleksandrov (1934) proposed the term of "paranecrosis" to describe the complex of these changes in the reversible phase. At the basis of paranecrotic changes they assume, is reversible denaturation of the protoplasm of the proteins which may be accompanied by the reconstruction of the protein molecules at the upset of the denaturation as well as at their reversion to the native state. Two conditions should be noted here: first, paranecrotic modifications are not specific to the action of various agents, and second, they are found throughout the entire thickness of the cytoplasm--in the chromatin of the nucleus, in the nucleole, and in other elements of the cell. The first of these conditions is confirmed also by genetic data, and indeed, by the fact that mutation develops under the effect of completely different agents. The development of mutation is not a specific reaction of the cell.

The second condition excludes the possibility of the direct action of the agent on gene modification and provides a basis for the thought that gene modification is a special event which takes places during the modification of the living substance of the cell as a whole system and its reaction to the effect of the environment.

If the reversible denaturation of the proteins is accompanied by a change in their structure, is it not possible to assume that in the process of their reversion from the reversible denatured (paranative) state to the native, that a restoration to their identical original state take place? It is difficult to accept the A priori that after each reversible alteration of proteins an exact copy of them is restored. Moreover, it is possible that this process of incomplete reversion to the initial state is of greater significance in the development of the living

systems than it was assumed to be prior to this time. But this belongs to the field of biochemistry which must provide biology with either a positive or negative answer. On the basis of cytophysiological and physiological data (teachings about parabiosis) we have now a sufficiently substantial basis to assume that the reversible modification of the cell under the impact of external conditions is a general biological law of the reaction of the living substance. We should not add our experimental data to this; however, inasmuch as special specificity is ascribed to the effect of the mutagenic factors on the sex cell, special experiments to learn the effect of X-rays and high temperature on the reaction modification of the living substance of the sex cells had to be carried out. For this purpose we utilized the changes in the adsorbing properties of the protoplasm which take place in relation to neutral red, considering it as an undoubtful intimate indication of substantial changes in the cells.

In these experiments adult male Drosophila were irradiated with X-rays or were exposed to a high temperature. Homogenous nonirradiated drosophila were used as control. In both and other tests and testis were severed and stained with neutral red while living (0.05 to 0.025%). Alcoholic extracts of the stains were then obtained from the stained testis. There were colorimetrated. On the basis of the intensity of the stain extracted from the testis of the experimental and control males, it was possible to determine the quantity of the stain absorbed by them. The experiments in regard to stain absorption by the testis were repeated some time after the cells were irradiated or exposed to high temperature. On the basis of the results obtained in these investigations the following conclusions were arrived at:

1) The reversible increase in the sorption properties of the cytoplasm of the sex cells after having been irradiated with X-rays or exposed to a high temperature is revealed by the method of vital staining;

2) The change in the sorption properties of the cytoplasm is an indirect indication of the reversible substantial changes which take place in the sex cells;

3) The tempo of the restoration (reversion) of the substantial changes in the cell to the initial stage--"norm"--is conditioned by: a) the depth of the irritation inflicted on the cell (effective dose); b) conditions under which restoration takes place;

4) If the action of the agent is parried the reaction of the organism as a whole then its effect does not reach the sex cells and does not increase their sorption properties.

These conclusions, based on a large number of experimental data, provide a basis for the assumption of the nonspecificity of the temporary and easily reversible substantial changes in the sex cells, changes which develop as a result of the unfavorable action of environmental factors on the cells.

Having organized into a definite system the accumulation of facts which were established in the course of the experimental study of the mutation process based on the physiological and cytophysiological laws, we have proposed a new working hypotheses for the explanation of the origin of mutations. Although, it is not yet complete, it explains better the accumulated fact and provides a better picture of the perspectives of investigation, than does the presently existing hypothesis in genetics --"Treffertheorie. "A gene model is represented as a complex protein molecule, according to the latter. Under the term of gene mutation is understood the reshuffling of the space positions of the atoms in this molecule, thus, changing its chemical properties. Chemically the gene is a complex, balanced molecule, with a structure of atoms in a definite arrangement. A change in the arrangement of the atoms in the molecule may be caused by a direct impulse from without caused by an electron or a light quantum. Simultaneously, mutation may be caused not only by an impulse from without, but also by accidental changes in the atom. The latter may take place without a strong induction agent, under normal conditions, depending on the strength of the interatomic bonds. Such variations may be sufficient for the regrouping of the entire system of atoms or part of it, and provide, on the basis of this hypothesis, the mutation effect of the gene.

It is not difficult to become convinced that the hypothesis of Treffeltheorie is attempting to explain the biological phenomenon of variability of the inherited peoperties of the organism as being purely the result of the physico-chemical change of the gene.

It should be noted, first of all, that little is known about the nature of the gene. As yet, there is no direct or indirect proof as to whether the gene is a materially individual unit of the chromosome. At the same time, however, it is known that a modifcation of one and same part of a chromosome induced by different methods, leads to an effect which is externally similar to that caused by a change in the character of the organism in the phenotype, and which is inherited by a number of generations. At the same time modifications of different parts of the chromosome produce different effects, pointing to the unequal value of the different parts, that is the selective reaction (discretion) of the chromosome.

No matter what the nature of the gene, it can be known only in the process of its modification. Therefore, leaving aside the discussion in regard to the nature of the gene, we think it more useful and important to examine the arguments for and against the previously proposed hypotheses, and to present the principles of our physiological hypothesis (paranecrotic) of the mutation process, from the point of view of an analysis of the general problem of the causes of variability.

The main arguments which support the Treffeltheorie are as follows: 1) the direct proportional relationship between the dose of ionization and the frequency of mutations; 2) the independence of this phenomenon from the wave length of the rays; 3) the direct action of external factors (electrons and light quantum in particular) on the

molecular structure of the gene and on the origin of mutations at the moment of their action (ionization), and absence of post effects; 4) rapidity with which gene mutation takes place at various temperatures with regard for the dependence of the chemical reaction on the tempera- ture (the rule of Vant-Hoffe). Not one of these arguments is suffi- ciently convincing for the acceptance of the indicated physico-chemical hypothesis.

Directly proportional dependence is not a specific rule in gene modification, inasmuch as the majority of the mutations which develop are conditioned by chromosome aberrations which may develop as a result of the simultaneous modification of the entire chromosome body or of all the chromosomes. In any case, this argues against any local changes in the chromosome. In addition, the direct dependence of the survival of monocellular organisms, the nonsegregation of chromosomes, chromosome aberrations, crossing over of drosophila males, the origin of roent- genomorphoses and, in our experiments, the increase in the sorption properties of the cytoplasm, on the ionization dose has been established. These modification may develop only if the alterations of the proteins take place throughout the entire thickness of the cell, or if the ac- tivity of the cell as an entire system has been disturbed. From the point of view of our hypothesis this phenomenon may be explained by the depth of the reversible injury inflicted on the cell by the dose of the agent; dependence of the frequency of mutations and other modifi- cations on the X-ray dose is the function of the degree of injury in- flicted on the cell.

The validity of the second argument is doubtful, for experiments carried out on neurospora (crassa) and drosophila had shown that under the effect of the same X-ray dose of different intensity (240 hertz a minute to 5400 hertz a minute) the frequency with which mutations de- velop is not the same.

The third argument--the relation of the direct effect of the factor on gene modification-- is not satisfactory even if only for the reason that the absolute majority of the agents (with the exception of radiation energy) act directly on the nucleus of the cell through the cytoplasm (chemical substances). Furthermore, as it was already indi- cated, fractional radiation with X-rays of the same dose induces more mutations and roentgenophormoses than does continuous radiation. This may be expected from the point of view of a physiological hypothesis, since the probable number of cases in which the incomplete reversion of the paranative proteins to the native occurs, is increased by frac- tional irradiation.

The attempt to present gene modification as being induced by a temperature typical of a chemical reaction is also not convincing, for mutations develop as a result of the effect of negative temperatures (minus 5 to minus 12 degrees C) on the organism with the calculation of Q_{10} being of no practical value; and if Q_{10} is to be taken into consid- eration it can be used mostly to reject the chemical nature of the gene modification. A large number of other facts may be cited which cannot in any way explain as "atomistic" the hypothesis under discussion. The mutation process is the reflection of a complex of intracellular

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substantial modifications which are conditioned by organism itself as well as by the combination of physiological processes of the cell. Just as the gene cannot act independently without the cellular system, so its modification cannot take place as an independent narrowly localized event. The mutation process is primarily a biological process.

In the light of what has been said here I shall take the liberty of presenting in brief form the propositions advanced by this hypothesis.

1. Mutations develop as the result of reversible substantial modifications of the cell under the effect of various irritants which vary in nature.

2. The effect of external conditions on mutation depends on their ability to remove the cell from the optimum state and produce paranecrotic modifications.

3. Gene mutations are the result of the incomplete reversion of reversibly altered cells from the paranecrotic phase to their native state during the process of renativation. Injury represents an extreme stage of irritation. The possibility for the rupture of the chromosomes and their break down into fragments arises if the paranecrotic changes have gone too deep, disrupting the forces of cohesion of the substrata of the fine structure of the chromosomes (upon the decrease of the dispersion ability of the colloids); chromosome aberrations develop as a result of the incomplete reparation of the injury.

4. Under the effect of the external conditions, the cell modifies its entire body, but as a result of the differential sensitivity of the nucleus and the plasma, and individual chromosomes or parts of chromosomes, it is possible to obtain with small doses a directed modification of some or other cell elements.

5. Mutation is the nonspecific reaction of the gene to the effect of an applied agent, and develops in conditions of a general cellular irritation. The cell reacts as a whole system to the irritant.

6. The frequency with which mutations develop is conditioned by the depth of the injury inflicted on the cell, and the cell's sensitivity to the changing conditions, which in their turn are determined by the capacity of the cell for substantial adaptation; the frequency with which mutations develop depends also on the ability of the cell to repair the injury, that is, the rapidity of the restoration processes following the cessation of the effect of the agent.

7. The directly proportional dependence of the frequency with which mutations develop on the dose of ionization is not a specific reaction of the gene modification; but is a general reaction of the cell reflecting the extent of its injury. Because of that, any changes which are accompanied by injury to the cell are in direct dependence on the dose of the agent.

8. The specificity of the effect of the agents on the mutation process is proportional to their injurious effect on the cell. The injurious effect by the agent is controlled by a large number of factors:

a) Physico-chemical properties of the agent able to induce the reversible alteration of the proteins;

b) The permiability of the agent; X-rays affect directly the sex cell while with most of the agents the effect is attained by means of a whole chain of internal processes in the organism;

c) The level of the metabolic processes; with a higher level of metabolism in the cell its sensitivity to injury increases; this may serve as an explanation for the higher mutability of the male sex of drosophila; a rise in metabolism linked with the maintenance of the native state of the cytoplasm under extreme conditions of deviation from the optimum, lowers the resistance of the cell to external effects and narrows its reparation capacity; organisms with a lower level of metabolism should be less mutable; [despite certain representations, it should be noted, that a rise in dominance, is apparently also more frequently conditioned not by an increase, but a decline in the level of metabolism, which leads to a rise in the resistance of the organism's tissues in the process of development.]

d) Intracellular correlation; the disturbance of the latter causes a rise in the sensitivity of the cell to a change in conditions, with the result that a rise in the mutability of the mutant lines, in hybrids as compared with the initial forms, and in lines structurally disturbed chromosomes takes place;

e) The adaptive reactions of the organism as a whole; the activity of its separate organs and systems which are the first to bear the entire burden of the harmful factor and which play a protective role for the sex system.

The enumerated factors which control the effect of the external medium on the substantial modifications of the cell, are only an insignificant part of the possible adaptive reactions which make up the "nature of the organism." Thanks to the presence of these factors the organism does not remain a passive "sack" of a living substance under changed conditions. The application of an agent to the cell of the organism is a complex process, and it is this which primarily creates the impression of a specific effect of individual factors on the mutation process.

M. S. Navashin (1933) was one of the first to arrive at the correct conclusion that "excessively different external effects applied to obtain artificial mutations produce essentially similar results; that the external factor does not directly influence the hereditary substance" (page 112). It is not the direct action which determins, to a considerable degree, the seeming specificity of the agents which induce mutations. Attempts have been recently made to select specific chemical substances which effect the modifications of the protein nature of individual genes; these attempts, however, will hardly prove to be successful for the solution of the problem, as it is impossible to obtain a direct and selective effect by acting directly on the gene with some definite chemical substances, by-passing the reaction of the cell as a whole.

Inasmuch as the problem of the specific effect of agents on the mutation process is one of the urgent problems of genetics, it is necessary to elucidate it from the point of view of the proposed hypothesis.

IV

Mutations, the hereditary development of modifications, are incidental in relation to the factors which determine the direction of selection, according to the accepted theory of evolution. Of the large number of incidental mutations which develop, only some may prove to be useful in the evolution of the species. It is but natural to ask, how could undirected hereditary variability develop in evolution. The explanation is that each gene is arbitrarily capable of change. However, if the evolution of the gene (more correctly speaking, the evolution of chromosome discretion) could be in one or another manner controlled by the factors of evolution, and this is a supposition of a number of hypotheses, which are attempting to explain the evolution of dominance, then, sooner or later, its modifications would acquire a form of an adequate reaction to a change in external conditions. We fail to notice this, however. The experimental attempts made by Goldshmidt and Wallace to induce directed mutations by the use of high temperature were not confirmed in the investigations conducted by many authors. These failures alone should have led the geneticists to the conclusion that the accidental modification of the gene cannot be explained from the point of view of the specificity of its modification. The modifications which take place in individual parts of the chromosome are the indirect response of the cell to the immediate effect of the agent from without. Undirected local modifications in the chromosome are accidental because they are not a result of the direct effect of the agent, but are the result of secondary processes which develop in the cell upon its irritation. The same reason, apparently, is at the basis of the fact that the mutation process is a nonspecific reaction of the cell to the external irritant.

If we accept the proposition that mutations are the result of the nonspecific reaction of the cell, then the problem of their undirected origin as regard to the factors which act on the cell becomes clear. The gene as such cannot enter into direct contact with the environment, for this is the function of the cell which enters into mutual relations with the environment.

In speaking, however, of the mutation process as a nonspecific reaction of the cell, the fact of the indices which characterize the specific effect of the external and internal factors on the mutation process cannot be denied. In citing the basic propositions of the physiological (paranecrotic) hypothesis of the mutation process, we have pointed out some of the basic moments which determine its specific side. When the cell is subjected to some action, it changes its entire complex physiological system. But inasmuch as the individual organs of the cell which make up its elements are different in their sensitivity, it is, but natural to assume that the substrata of these elements will undergo various changes during the phase of injury, as well as during the phase of reparation.

The authors of the theory of paranecrosis (Nasonov and Aleksandrov, 1940) particularly point out that although "paranecrotic changes" are characteristic of all the indicated structures (cytoplasm, chromatin of the nucleus, nuclear membrane, and so forth)--M. L.), they are manifested by these structures in a different manner and not with uniform acuteness. The succession of the manifestations of paranecrotic indices in different parts of the cell may vary, depending on the nature of the cell itself, as well as on the properties of the irritant (Italics are mine). The succession with which paranecrotic indices in different parts of the cell are manifested, point, first of all, to the difference in the sensitivity of the components which make up this part to the cell irritants.

The difference in the sensitivity of the cell parts to the damaging effect of the external factors may create the conditions for directed derivation of mutations. The effect of colchicine, acenaphtlene, and others served as vivid example of it.

Thanks to the fact that colchicine increases the viscosity of the cytoplasm, the formation of the spindle during the division of the cell is inhibited. Polyploid cells are formed as a result of the incorrect distribution of chromosomes in the dividing cell. There is no doubt, that just as a single agent in different doses can act on a cell, so can the use of different agents find its application in the cell. In the investigations which were carried out to determine the effect of chemical substances on the mutation process, we pointed out (Lobashev and Smirnov, 1934, 1937) that alkaline and acid media affect differently the intracellular (mutation) modifications. The action of acetic acid on the cell is accompanied by a rise in the viscosity of the protoplasm. The action of ammonium is characterized by the liquefaction of the protoplasm. A study of the effect of these two agents on the mutation process--the development of lethal mutation and nonsegregation of chromosomes--has shown that acetic acid does predominantly cause the nonsegregation of the chromosomes, and only very weakly induces lethal mutations. Ammonium produces a reverse picture: it is little effective in increasing the nonsegregation of chromosomes, and induces lethal mutations. These experiments provided a basis for the supposition that the origin of marked and small chromosome aberrations is linked, first of all, with the liquefaction of the colloid, while the nonsegregation of the chromosome--with a rise in the viscosity of the cytoplasm (structurization of the chromosomes).

Many facts which have been gathered at the present time confirm this supposition. In a series of works with X-rays it was established that small doses of ionization produce predominantly localized changes within the chromosome. An increase in the dose of ionization increases the number of ruptures in the chromosomes, leading to the formation of chromosome aberrations (inversions, translocations, and others). V. C. Sakharov (1938) compared lethal mutations which develop under the influence of high doses of X-rays with those produced by changes of chemical agents and temperature. It was found that most of the mutations which develop under the influence of high doses of ionization are linked

with chromosome abberrations, while those produced by the action of chemical agents are connected with local changes. These and similar data concur with the supposition that localized modifications in the chromosome are connected with the liquefaction of the colloid in the first phase of the injury.

In determining the symptoms of the paranecrotoc changes in the cell on the basis of the enormous quantity of factual data available, Nasonov and Aleksandrov proved that the increase in the viscosity of the cytoplasm is sometimes preceded by some decrease in viscosity, that is, the liquefaction of the colloid of the cytoplasm proteins. It is possible that small doses of ionization, and the effect of chemical substances, temperature, and other agents are in a number of cases capable of evoking only the first phase of a reversible injury--the liquefaction of the cytoplasm colloid--an effect which frequently may be connected with the predominant development of local changes in the chromosome of a type of point mutations. The intensification of the effect of the external factor leads to a deeper injury of the substrata of the cell, which increases the frequency of the development of chromosome reorganizations.

The indicated connection between the type of mutations which develop and the colloidal modifications of the cytoplasm proteins under the impact of damaging agents is hardly an accidental coincidence. This connection is a natural phenomenon which is subject to further investigation. This connection, of course, is considerably more complex than pictured here. However, with a reversible alteration of cytoplasm proteins as a base for the development of mutations, the problem of the specificity of the mutations which develop becomes explainable in physiological terms.

In characterizing the specific aspects of the effect of the agents on the mutation process, we should not fail to pay attention to the fact that the agents affect differently the character of the reversibility during the process of the denaturation of the proteins. This aspect is particularly important, for we proceed on the premise that the development of the mutation is not a part of the phase of the injury or irritation of the cell, but is a part of the phase of restoration--the process of the incomplete reparation of the substantial changes. The problem of the reversibility of the injured substratum of the cell is of great significance to the interpretation of the nature of the mutations which develop. It is known, that in one case all that is necessary to return the changed substratum to its initial state is to simply remove the cause which inflicted the injury (homodromic reversibility), while in another case it is necessary to subject the changed product to additional processing (heterodromic reversibility) to return the substratum to a normal state. Other types are encountered in addition to the above-mentioned two.

It is interesting to note that inasmuch as quantitative and qualitative differences exist between the development of mutations by the action of radiation energy on one hand, and the temperature or chemical substances on the other hand, it is these two types of agents which considerably differ in their effect on the coagulation of the proteins.

The withdrawal of heat from the protein causes a halt in its coagulation; when roentgen rays or radium rays are withdrawn the coagulation n process continues for a considerable period of time. The specificity of the effect of the agents on the character of the reversible alteration of the proteins in the cells system cannot but reflect on the development of one or another type of mutations.

If the development of mutations depends on the alteration of the cytoplasm proteins, the question then arises: to what extent does the level of the cell's metabolism, the metabolic process, affect the sensitivity of the proteins to the reversible alteration which controls the mutation process? It is obvious, that exact data in this sphere are difficult to cite. Many facts, however, are available which indirectly point to the dependence of the mutation process on the level of metabolic processes and the state of the native proteins. As an illustration I shall cite several examples.

The process of the aging of seeds in a quiescent state is marked primarily by the dehydration of the protoplasm, a typical sign of denaturation. A number of authors (Navashin, Shtubbe, and others) established that seed aging is accompanied by an intensification of the mutation process. The process of the aging of seeds in itself may be regarded as a spontaneous denaturation, the result of the dehydration of the proteins of the cytoplasm as well as an act of the artificial inhibition of development, an act which probably is not normal to the protein system of a living substance; with the return of proper and favorable conditions for development, the coagulation of the proteins which takes place in the course of aging becomes reversible. The incomplete reversion of the reversible denatured proteins to their native state may serve as a cause for the development of mutations.

Further, there can be little doubt, that the metabolic processes in quiescent seeds are depressed and their course is considerably weaker than that in the germinating seeds. Investigations conducted by a number of writers established that sublethal doses of X-ray for the first are 14 to 17 times greater than for the germinating seeds. In order to obtain a similar percentage of mutations by Roentgen rays in quiescent as well as in germinating seeds, a higher dose of radiation is required for the first than for the second. It should be noted, at the same time, that the mere swelling of the seeds when their development if delayed, does not increase their sensitivity to the action of radiation energy. Therefore, the high sensitivity of the protoplasm proteins of germinating seeds cannot be explained as a result of their simple hydration, but only as a result of a rise in the metabolic processes. It is thus, that the metabolic level is one of the factors which controls the effect of the agent on the reversible denaturation of the proteins; the higher the metabolic level the greater the sensitivity of the proteins to alteration.

It is necessary to mention here that the phenomenon of the higher rate of metabolism can be interpreted not as a cause, but as a result of the higher sensitivity of the protoplasm proteins; a higher metabolic level is required in order to retain them in their native state. It is clear that it is not possible to differentiate between the cause and the effect in the substantial sensitivity without carrying out direct

experiments. If the causes of mutations are analyzed from this aspect, then no further details are needed. It is important now to emphasize the dependence of the mutation process on the sensitivity of the cell's substrata to external irritants.

Attention has long been called to the fact that the frequency with which mutations develop differs in the male and female sexes. The male sex in drosophyla is more mutable than is the female sex. The percentage of sterility in the male sex when subjected to radiation by X-rays is considerably greater than that in the female sex. A similar picture is noted in the study of roentgenomorphoses and modificational variability. At the same time it was noted that the sensitivity of the embryonic tissues in the male drosophila to damaging factors is higher than those of the female. General vitality of the male organism is lower than that of the female. The impression is formed that the higher mutability of the drosophila males is only a special reflection of a more common law, that is, the manifestation of the greater sensitivity of the male cell tissues to damaging factors. An investigation of the intensity of metabolism revealed that it is considerably higher in males than it is in females. The conclusion of this juxtaposition is that at the basis of the high sensitivity of the male cells to the damaging effect of external factors is the higher level of metabolism which lowers the threshold of injury, and which hastens the manifestations of paranecrotic changes in the cell, thus, causing the rise in the frequency of the development of mutations. If this conclusion cannot be regarded as being fully proved, it still provides an explanation for a whole group of different and not connected facts which generally remained unclarified. In any case, the clarification which we have provided for a whole group of phenomena makes it possible to form a concrete representation of the fact that substantial sensitivity is one of the basic factors which characterizes the nature of the organism, and which controls and to some degree limits the reaction of the living substance to external irritants. Therefore, the differences in the mutability of the sexes, in their modificational variability, and a number of other distinguishing characteristics in the sexes owe their origin to the difference in the sensitivity of the male and female tissue cells to injury; this sensitivity is conditioned by the level of the metabolism and the difference in the substantial sensitivity which creates the conditions for the specific manifestation of the reaction of a living substance.

The biological causes, which are at the basis of the differential sensitivity of the native protoplasm proteins of the cells of different organisms and even cells of one and the same tissue, vary. It is not possible to analyze them here. Nevertheless, we shall dwell on one of the phenomena which is closely linked with the study of the mutation process.

A change in external conditions forms a powerful factor in the modification of the reaction of a living substance. Facts which demonstrated that in an insect a rise of temperature causes an increase in oxygen absorption and a rise in metabolism, have already been cited.

However, not only factors working from without, but internal causes as well may be the cause of a rise in metabolism and the increase in substantial sensitivity. Thus, for instance, critical stages in the development of the organism are accompanied by a rise in metabolic processes; the same is noted in hybrids, triploidite organism, and in crossed organisms. It is possible that the disturbance of the intracellular correlation may be on of the internal causes which leads to a higher sensitivity of the cell's cytoplasm proteins to external irritants. Genetic facts are in full agreement with this supposition. The frequency of mutation of a gene in the plasma of a plant of the same specie is considerably lower than that of a gene of one specie in the plasma of another specie. The hybridization of different species, or genetically different lines, can also lead to an increase of (pointed) mutations as well as of chromosome aberrations. Numerous cytological investigations established that hybrydization frequently produces pathological pictures of the behavior of chromosomes in meiosis. The rise in the mutability of the hybrid cells cannot be explained from the point of view of the autonomous modification of the gene. Physiological conditions in the hybrid organisms which differ from those in the initial parental forms are responsible for the increase in mutational variability. What is more probable is that in the process of hybridization, protoplasm proteins of cells which are close but not identical and which evolved from different organisms are combined; this leads to a rise in their sensitivity in the new and uncorrelated cell system. Thanks to this, the threshholds of cellular injury are lowered, and the denaturation processes are easily induced by external conditions, as well as by spontaneous reactions in the process of development; the result is a mutational variability, in accordance with the scheme of the proposed hypothesis.

There is doubt that the role which the reaction of the protoplasm protein system in the cell plays in the control of the action of external factors is more complex, and we provide only the first indication of that scheme which is implemented in the process of the interrelation of the "nature of organism" and the "nature of conditions."

V

In the experimental investigations of the mutation process, phenomena are frequently encountered in which the factor which is applied to induce mutations fails to act. On the basis of this phenomenon, external factors are divided into those which are able to induce mutations, and those which are unable to do so. Such division, however, is not always justified; the fact that an agent when applied fails to produce mutation is not a sufficient basis for the denial of its ability to induce mutations. From the point of view of the hypothesis which is being discussed, even a mechanical injury to the cells may prove to be sufficient to induce mutations. Different reasons may be responsible for the failure of an agent to act: impenetrability of the cellular membranes,

dissociation of the molecule of a chemical substance which is applied
as an agent of mutation, and others. In addition, there are processes
in the organism which control the effect of external factors on the sub-
strata of the cell tissues. I have in view the activity of the organism
as a whole system, the adaptive reactions of the organism, the functions
of the nervous system, and others which to a known degree, serve as a
shock absorber, a buffer which protects the organism from substantial
modifications of the cells of which it is composed. This applies in
particular to the sex system and the sex cells which should be maximally
protected from accidental fluctuations of the environmental factors by
the adaptive reactions of the organism as a whole. To illustrate this,
I shall cite several examples of results which we hav experimentally
established.

A number of authors attempted to induce mutations with the help
of anesthetics. The results were negative. There is that possibility,
that when drosophila are anesthesized by ether, the ether would paralyze
the nervous system limiting the effect of the anesthetic, and precluding
its reaching the sabstrata of the sex cells. P. V. Makarov (1938) proved
that with certain doses of the anesthetic it is possible to attain only
a general anesthesia of the organism without anesthesizing the cells.
In order to explain this we Lobashev and Korenevich) applied the method
of vital staining of the drosophila gonads, a method which was already
described.

Had the action of the ether reached the drosophila gonads, we
should have attained a vital staining of the anesthesized drosophila of
greater intensification than that of fly gonads which were not anesthesized.
Experiments have shown that anesthesia applied at room temperature even
if only for periods of three to four hours has no effect on the substan-
tial modifications of the sex cells. Therefore, the negative effect of
anesthesia on the mutation process can be explained simply by the fact
that it does not reach the sex cells. The paralysis induced by the anes-
sthetic is limited to the suppression of the nervous system functions and
does not affect the sex cells.

On the basis of these experiments it became necessary to determine
in what manner does the nervous system itself play the role of the "con-
troller" of the other factors, temperature in particular, which affect
the drosophila gonads. For this purpose the flies were divided into two
groups in each experiment: one group was subjected to general anesthe-
sia, and the other was maintained in an active state. Both groups were
simultaneously exposed to the effect of a high temperature of 30 or 33
degrees C. If the exclusion of the nervous system with the help of gen-
eral anesthesia opens the way for the effect of high temperature on the
gonads, than the injury to the latter caused by the high temperature will
be more severe, and the deposit of the stain will be greater as compared
with the control. Numerous experiments fully confirmed this. The same
temperature, applied to the specimens which were placed under general
anesthesia and those maintained in an active state, had a more injurious
effect on the sex cells of the former than on sex cells of the latter.

The latter could parry the harmful effects of the given temperature by means of the adaptive reactions which are inherent in them. I shall not elaborate on the other interesting and important details of these experiments, but the significance of the regulatory processes of the organism as a whole in limiting the effect of the external conditions on substantial modifications of the sex cells are obvious from what has already been stated. The regulatory processes of the organism, in limiting the effect of external conditions, can lead to their specific manifestation in inducing mutations.

The physiological (paranecrotic) hypothesis of the mutation process presented here is built on genetic data reduced to a system which regards the mutation process from the point of view of cytophysiological laws and a biological tracing of the causes of variability. It is as yet not completed in many details, and requires further development; however, in its present stage it already serves as a working hypothesis which broadens the perspective investigations and makes it possible to overcome a number of mechanical representations in the explanation of the mutation process.

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